



Verification of Chemical Warfare Agent Exposure in Biomedical Samples using Mass Spectrometry

J.R. Smith, B.R. Capacio, W.D. Korte, & M.T. DeLion

Analytical Chemistry Team

U. S. Army Medical Research Institute of Chemical Defense

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Technical Bulletin Medical 296 (TB MED 296)

- MRICD Internet Site:

<http://usamricd.apgea.army.mil/>

- Under Related Information:

- *TB MED 296*: Analytical techniques to detect exposure to toxic chemical agents in urine or blood samples.
- *SOP for Biomedical Samples*: Standard operating procedure for obtaining, handling, and shipment of biomedical samples.



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Technical Bulletin Medical 296 (TB MED 296)

<u>CW Agent/Degradation Compound</u>	<u>Method</u>
Sulfur Mustard (HD)/TDG	GC/MS
Sarin (GB)/IMPA	GC/MS
Soman (GD)/PMPA	GC/MS
Cyclo-Sarin (GF)/CMPA	GC/MS
Cholinesterase Inhibitors	Colorimetric
Cyanide (CN)	Colorimetric



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Human Exposure to OP Nerve Agents

Where do we look? What do we look for?

Urine

Reactions of OP's with:



GB > IMPA
GD > PMPA
GF > CMPA
EMPA
EDMPA

Tissue

Reactions of OP's with *Tissue AChE*

Blood

Reactions of OP's with:

RBC

Plasma/Serum

Plasma

AChE

BuChE

Protein/Albumin



measure inhibition level of
AChE and/or BuChE

phosphylated tyrosine

release of bound hydrolysis
products (IMPA, MPA)

fluoride reactivation of
phosphylated BuChE

release of bound
hydrolysis products
(IMPA, MPA)

Cholinesterase Inhibition

TB MED 296

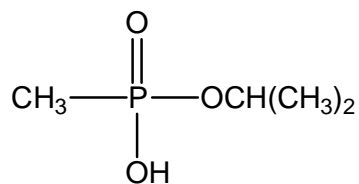
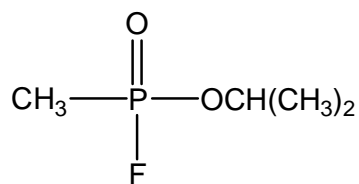
- RBC AChE, Plasma BChE
- 10 μ L of blood, finger prick
- Colorimetric Assay
- Field Portable System
- Test-Mate Kit (Patrick Eberly - EQM Research)



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OP Hydrolysis Reactions

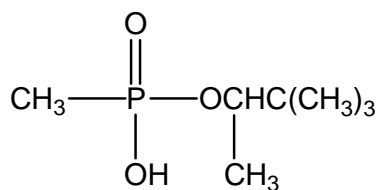
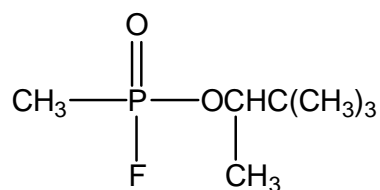
Sarin (GB)



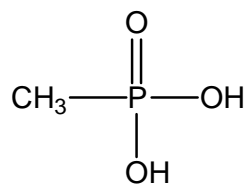
IMPA



Soman (GD)

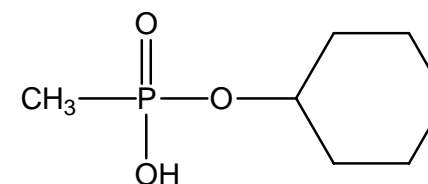
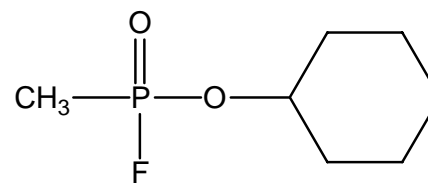


PMPA

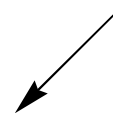


MPA

Cyclosarin (GF)



CMPA



Analysis of OP Hydrolysis Compounds

TB MED 296

- **Analysis for IMPA, PMPA, CMPA**
- **Deuterated IMPA and PMPA for Internal Std.**
- **1 cc urine, SPE cartridge cleanup**
- **Derivatization using PFB-Br**
- **Analysis using Electron Impact GC/MS**
- **Selected Ion Monitoring, low ng/ml sensitivity**

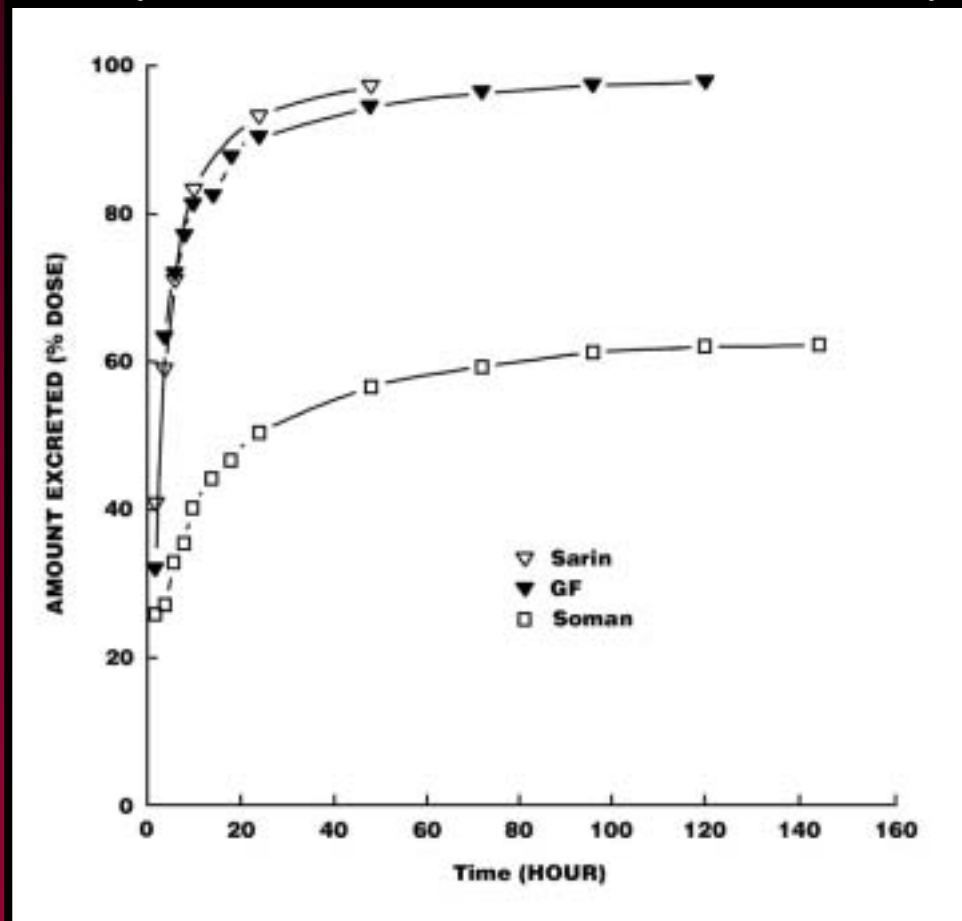


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Analysis of OP Hydrolysis Compounds

TB MED 296

Urinary Excretion (% dose) of GB, GF, and GD Hydrolysis Compounds vs. Time



Animal: rat

Agent: GB, GD, GF

Dose: 75 $\mu\text{g kg}^{-1}$ (sub-lethal)

Admin. route: subcutaneous

M.L. Shih, J.D. McMonagle, T.W. Dolzine, &
V.C. Gresham,

J Applied Toxicology **14** (3), 195-199 (1994)



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Analysis of OP Hydrolysis Compounds

- **W.J. Driskell, L.L. Needham, D.B. Barr (CDC) & M.L. Shih (MRICD)**
- **Urinary metabolites of GA, GB, GD, GF, and VX**
- **Isotope dilution GC/MS-MS**
- **Derivatization using diazomethane**
- **Single Reaction Monitoring, low ng/ml sensitivity**
- **J. Analytical Toxicology, Jan/Feb 2002**



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Human Exposure to OP Nerve Agents

Where do we look? What do we look for?

Urine

Reactions of OP's with:



IMPA
PMPA
CMPA
EMPA
EDMPA

Blood

Reactions of OP's with:

RBC

Plasma/Serum

Plasma

AChE

BuChE

Protein/Albumin



measure inhibition level of
AChE and/or BuChE

phosphylated tyrosine

release of bound hydrolysis
products (IMPA, MPA)

fluoride reactivation of
phosphylated BuChE

Tissue

Reactions of OP's with *Tissue AChE*



release of bound
hydrolysis products
(IMPA, MPA)

Analysis of Phosphylated Proteins

Analytical Approach:

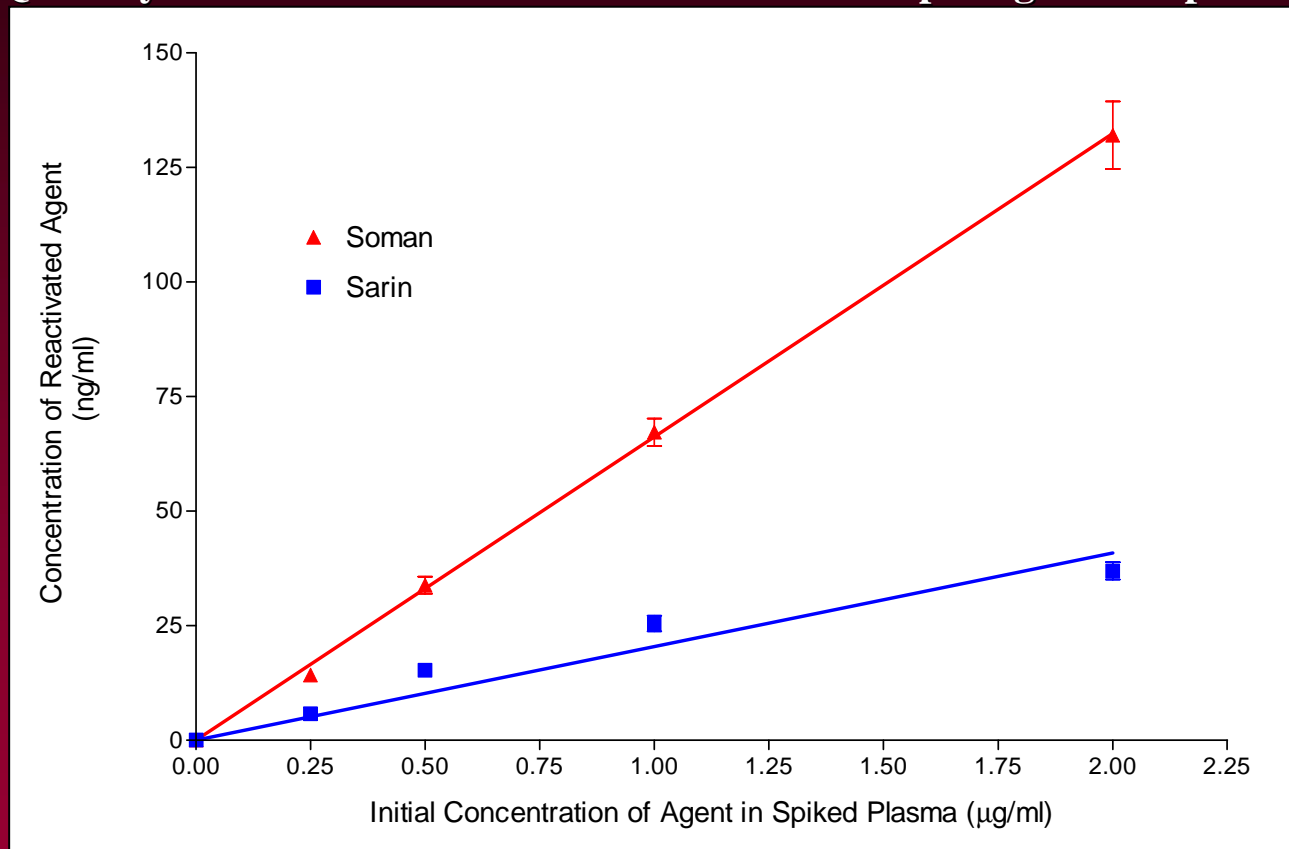
- Incubate sample with 0.7 M KF at 40°C
- C18 solid phase extraction cartridge
- Elute analyte from cartridge using ethyl acetate
- Dry eluent
- GC-MS analysis in SIM mode
- M. Polhuijs, J.P. Langenberg, & H.P. Benschop,
Toxicol. Appl. Pharmacol. 146 (1997)



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Analysis of Phosphylated Plasma Proteins

Quantity of reactivated GB and GD formed after spiking human plasma



Samples heated at 40°C for 2 hrs.

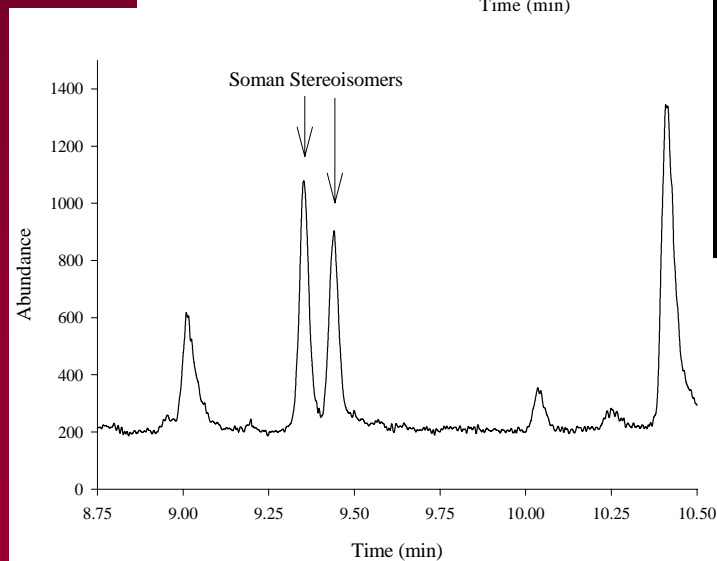
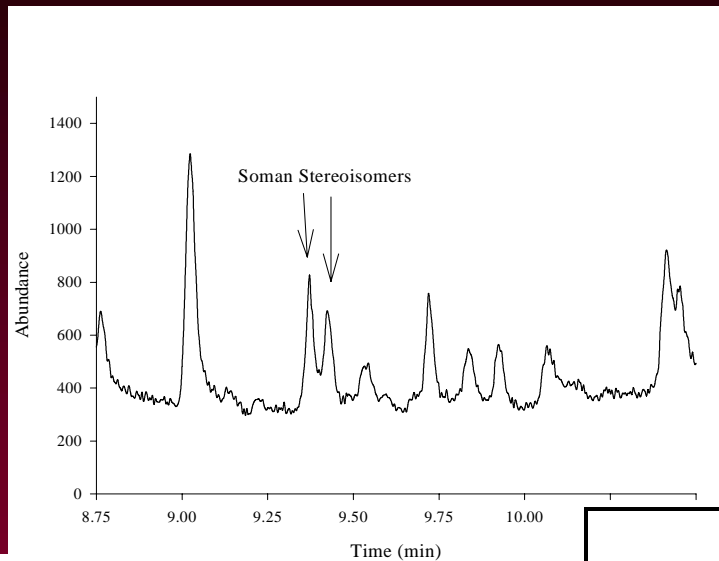
Each data point represents the average of 6 samples.

CV's for each data point range from 5 to 8 %.



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Analysis of Phosphylated Plasma Proteins



SOMAN CONC		
PLASMA	ng/ml	nM
Animal C	11	61
Animal D	4.5	24

Animal: guinea pig

Agent: GD

Dose: 0.2 LD₅₀
(M-F x 4 weeks)

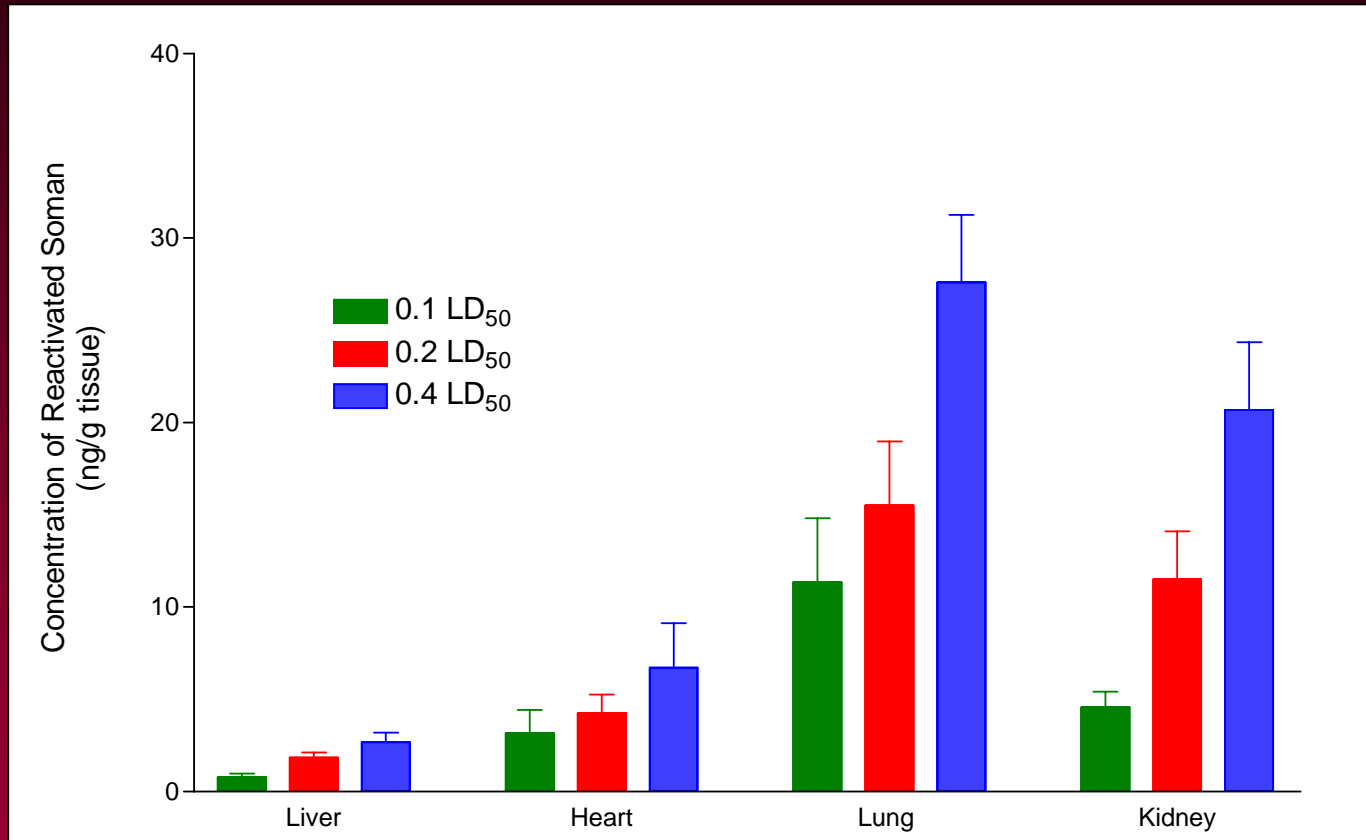
Admin. route:
subcutaneous

Sample: plasma
(collected 72 hours
after last GD dose)



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Analysis of Phosphylated Tissue Proteins



Animal: guinea pig

Agent: GD

Dose: 5 consecutive days at listed dose

Admin. route: subcutaneous

Sample: tissue (collected 4 hours after last GD dose)

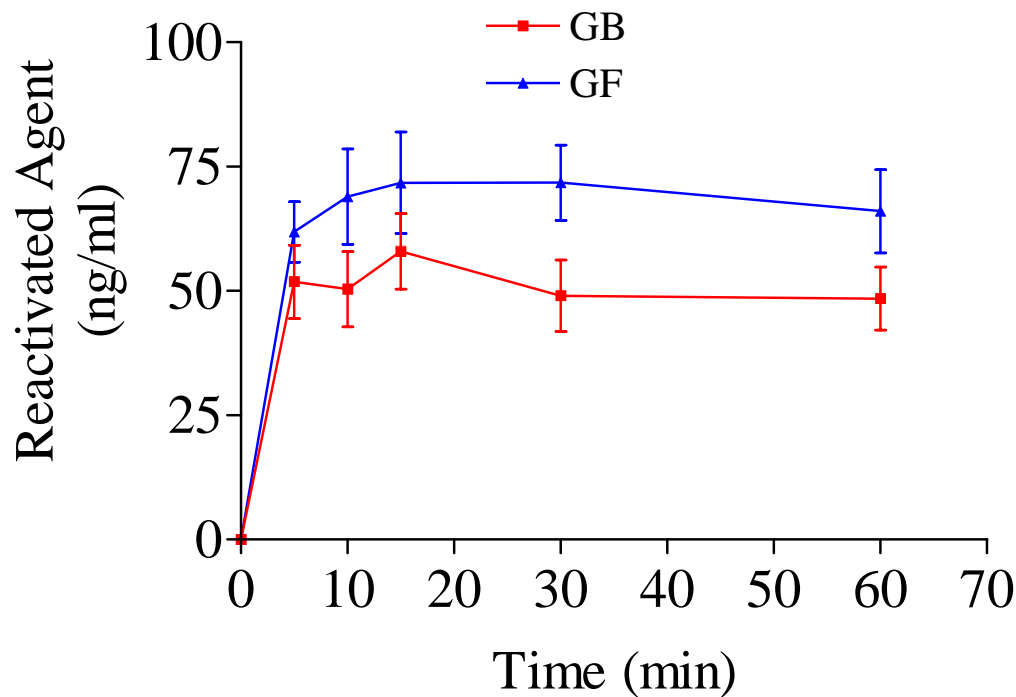
Number: each data point average of 8 samples



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Analysis of Phosphylated Plasma Proteins

Time-Course of Fluoride Reactivated GB and GF



Animal: guinea pig

Agent: GB,GF

Dose: 1 x LD₅₀

Admin. route:
subcutaneous

Sample: plasma

Number: each data
point average of 8
samples



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Wednesday Poster Presentations

**Time Course of the Nerve Agents Sarin (GB) and Cyclosarin (GF)
in Plasma and Kidney of Exposed Guinea Pigs**

**B.R. Capacio, J.R. Smith, B.E. Dusick, W.D. Korte,
J.H. McDonough, and T.-M. Shih**



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Human Exposure to Sulfur Mustard (HD)

Where do we look? What do we look for?

Urine

Reactions of **HD** with:

H₂O

↓
TDG
TDGSO

Glutathione

↓
cysteine conjugates
↓
β-lyase metabolites

DNA

↓
N7-guanine
adduct

Skin

Reactions of **HD** with:

DNA

→ N7-guanine adduct

Keratin

→ Alkylated glutamic & aspartic acids

Blood

Reactions of **HD** with:

RBC's

↓
N-terminal valine
of hemoglobin
Alkylated histidines
of hemoglobin

Plasma

↓
Alkylated cysteines
of albumin
Free carboxylic acid
sites of proteins

DNA

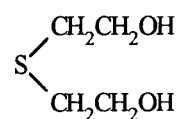
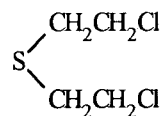
↓
N7-guanine
adduct

Hair

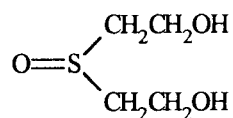
Fatty Tissue

Unreacted HD

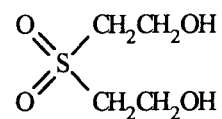
Mustard (HD)



TDG



TDG sulfoxide



TDG sulfone

Hydrolysis and Oxidation of Sulfur Mustard

Analysis of an HD Hydrolysis Compound

TB MED 296

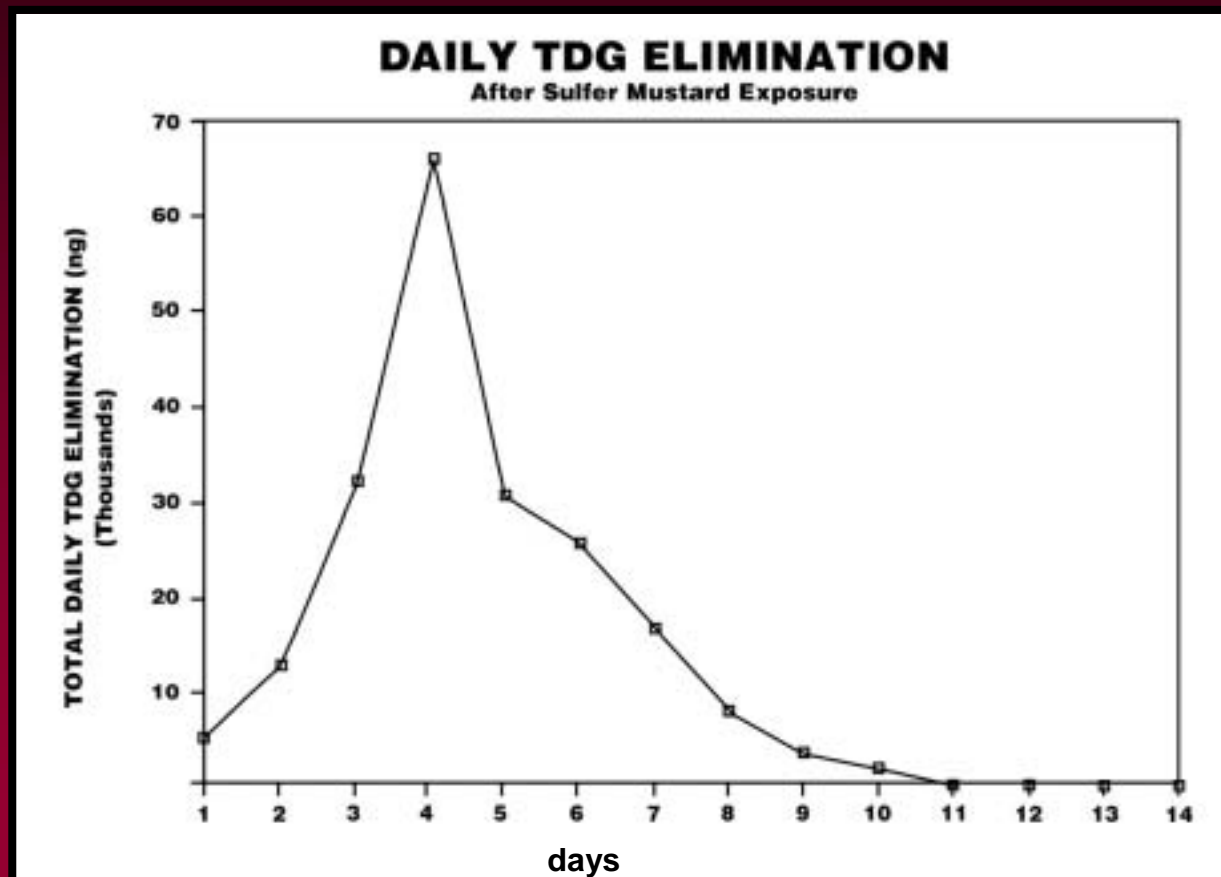
- **Analysis for TDG**
- **Deuterated TDG for Internal Standard**
- **1 cc urine sample**
- **Derivatization using HFBA**
- **Analysis using Electron Impact GC/MS**
- **Selected Ion Monitoring, low ng/ml sensitivity**



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Analysis of an HD Hydrolysis Compound TB MED 296

Thiodiglycol Urinary Excretion following an Accidental Human HD Exposure



E.M. Jakubowski, F.R. Sidell, R.A. Evans, M.A. Carter, J.R. Keeler, J.D. McMonagle, A. Swift, J.R. Smith, & T.W. Dolzine,

Toxicology Methods **10**, 143-150
(2000)



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Human Exposure to Sulfur Mustard (HD)

Where do we look? What do we look for?

Urine

Reactions of **HD** with:

H₂O

↓
TDG
TDGSO

Glutathione

↓
cysteine conjugates
↓
β-lyase metabolites

DNA

↓
N7-guanine
adduct

Skin

Reactions of **HD** with:

DNA

→ N7-guanine adduct

Keratin

→ Alkylated glutamic & aspartic acids

Blood

Reactions of **HD** with:

RBC's

↓
N-terminal valine
of hemoglobin
Alkylated histidines
of hemoglobin

Plasma

↓
Alkylated cysteines
of albumin
Free carboxylic acid
sites of proteins

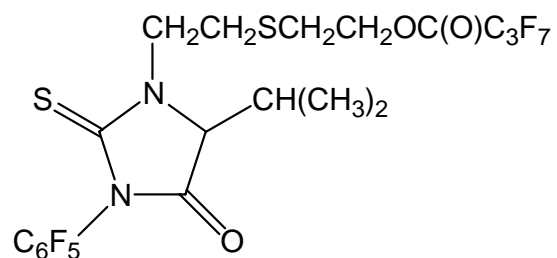
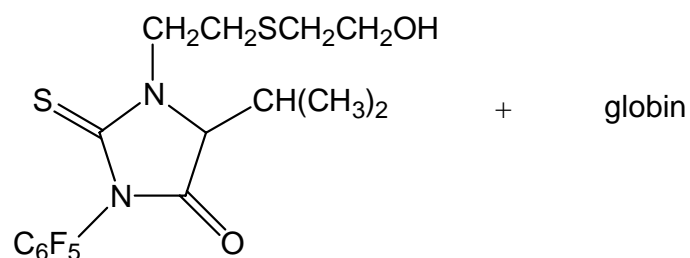
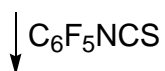
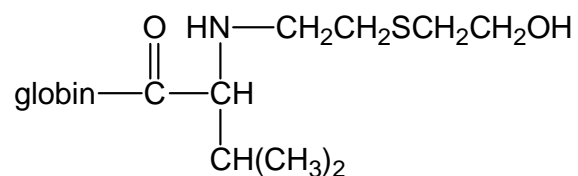
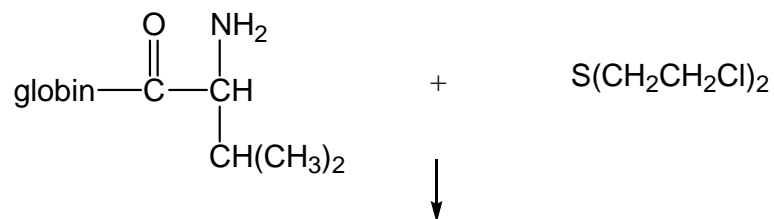
DNA

↓
N7-guanine
adduct

Hair

Fatty Tissue

Unreacted HD



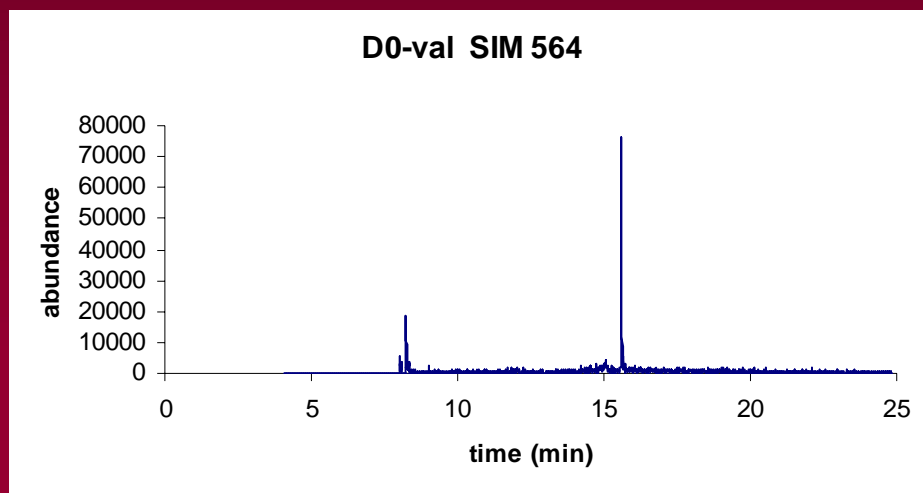
Reaction of N-terminal
valine of globin with HD

Modified Edman
degradation of N-
terminal valine containing
HD adduct (HETE)

Derivatization of HETE
hydroxyl group


Analysis of HD Adduct of Globin

- Method successfully reproduced at MRICD
- GC/MS method using negative ion chemical ionization
- Reproducibility and sensitivity of assay (100 nM) were similar as to those achieved at the TNO



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Payoffs

- **Benefits to the warfighter:**
 - Increased chance of verifying low-level incident
 - Extend time period: from CW event  to collection of biomedical sample
- **Benefits to the Chem-Demil community:**
 - Occupational health surveillance of military, civilian, & contractor



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Payoffs

- **CW Treaty Compliance:**
 - DTRA Chem/Bio Arms Control Joint Method Working Group
- **Homeland Defense:**
 - Center for Disease Control (CDC)
 - State/Regional Public Health Laboratories



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Acknowledgements

MRICD:

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Shannon Shuman

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John Schlager

Mike Jakubowski

Elvis Price

Brandon Dusick

Joe McMonagle

LTC Harry Slife

LTC Tim Adams

LTC Brian Lukey

LTC Ted Dolzine

COL Gennady Platoff

Other agencies:

Cathi Hoefler, Larry Pollack, Margo Jackisch (DTRA)



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United States Army Medical Research and Material Command

United States Army Medical Research Institute of Chemical Defense

United States Army Medical Research Institute of Chemical Defense



“Research for the Warfighter”



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